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Brief Report

Both exposure to a novel context and associative learning induce an upregulation of AKAP150 protein in mouse hippocampus

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Abstract

A-kinase anchoring protein 150 (AKAP150) is a multi-enzyme signaling complex that coordinates the action of PKA, PKC, and PP2B at neuronal membranes and synapses. We measured levels of AKAP150 protein in the hippocampus 6 h after training mice in a contextual fear conditioning paradigm. In contextual fear conditioning mice learn to associate a context with a footshock presentation. Mice were divided in four experimental groups with different training protocols: naive, no footshock exposure, immediate footshock exposure, and footshock 3 min after exposure to the context. We found that AKAP150 protein levels were increased upon exposing mice to the novel context independent of the training protocol. However, when the animals were habituated to the experimental context, only mice that learned to associate the context with the footshock showed an upregulation of AKAP150. We suggest that upregulated levels of AKAP150 contribute to processing the exposure to a novel context and associative learning.

Keywords: A-kinase anchoring protein; Contextual fear conditioning; Memory

The phosphorylation of intracellular proteins is a general mechanism used to control diverse cellular processes that occur in response to extracellular signals. Since many protein kinases and phosphatases are widely distributed throughout the cell and often exhibit a broad substrate specificity, additional mechanisms are used to contribute to the organization and specificity of signal transduction pathways by favoring the accessibility to certain substrates. The subcellular localization of cAMP-dependent protein kinase (PKA) is tightly controlled by a family of A-kinase anchoring proteins (AKAPs) (Rubin, 1994). AKAPs have been shown to interact with a number of signaling proteins, allowing for the localization and segregation of multi-enzyme signaling complexes. This capacity of AKAPs to coordinate multi-enzyme signaling complexes is very well exemplified by the neuronal AKAP79/150 family of anchoring proteins. This family consists of three structurally similar orthologs: bovine AKAP75, murine AKAP150, and human AKAP79 (Carr, Stofko-Hahn, Fraser, Cone, & Scott, 1992). AKAP79/150 targets PKA, protein kinase C (PKC), and protein phosphatase 2B (PP2B/calcineurin) to the same intracellular locus. At the postsynaptic membrane of glutamatergic synapses, this AKAP79/150 complex is recruited to NMDA and AMPA glutamate receptors by postsynaptic density (PSD)-95 family membrane-associated guanylate kinase (MAGUK) scaffold proteins (Colledge et al., 2000). It was found that this multi-enzyme signaling complex plays an important role in coordinating changes in synaptic structure and receptor signaling functions underlying synaptic plasticity (Dell'Acqua et al., 2006). AKAP150 mRNA was shown to be upregulated in the hippocampus 3–12 h after the induction of LTP, a long-lasting enhancement in synaptic efficacy (Genin et al., 2003). In addition, pharmacological inhibition of PKA anchoring to AKAPs impaired late-phase LTP in hippocampal slices (Huang, McDonough, & Abel, 2006). Since synaptic plasticity is widely considered to

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be the cellular mechanism that underlies information storage in the brain, an important role of AKAP79/150 in learning and memory processes may be expected.

To date the only evidence for a role of AKAP79/AKAP150 in learning and memory processes came from a study by Moita, Lamprecht, Nader, and LeDoux (2002). They reported that inhibition of PKA anchoring to AKAP150 in the rat lateral amygdala impairs memory consolidation of auditory fear conditioning (Moita et al., 2002).

To investigate if learning is associated with changes in the expression of AKAP150 we assessed in the present study the expression of AKAP150 in the mouse hippocampus after a single training session in a contextual fear conditioning paradigm. Contextual fear conditioning is a hippocampus-dependent form of associative emotional learning.

All experiments were performed with 9–12 weeks old male C57BL/6J mice (Harlan, Horst, The Netherlands). The procedures concerning animal care and treatment were in accordance with the regulations of the ethical committee for the use of experimental animals of the University of Groningen.

Upon arrival mice were individually housed in standard macrolon cages and maintained on a 12 h light/dark cycle (lights on at 7.30 a.m.) with food (hopefarm® standard rodent pellets) and water ad libitum. A layer of sawdust served as bedding. The animals were allowed to adapt to the housing conditions for 1–2 weeks before the experiments started.

Contextual fear conditioning was performed in a plexiglas cage (44 × 22 × 44 cm) with constant illumination. The training (conditioning) consisted of a single trial. The mouse was exposed to the conditioning context for 180 s followed by an electric footshock (ES; 0.7 mA, 2 s, constant current) delivered through a stainless steel grid floor. The mouse was removed from the fear conditioning box 30 s after ES termination to avoid an aversive association with the handling procedure (P-ES group, Fig. 1a). The specificity of induction of fear was controlled by groups consisting of mice exposed to the context only (No-ES group) or to an immediate ES (ES-P group) (Fig. 1a). This latter group was introduced in order to discriminate between the fear response representing a conditioned response to the context, from an unconditioned response to the ES and possible sensitization induced by the ES.

Memory tests were performed 24 h after fear conditioning. Contextual fear memory was tested in the fear conditioning box for 180 s without ES presentation. Freezing, defined as the lack of movement except for respiration and heart beat, was assessed by a time-sampling procedure every 10 s throughout memory tests. In addition, mean activity of the animal during the training and retention test was measured with the Ethovision system (Noldus, The Netherlands).

A separate set of animals was used to measure the expression levels of AKAP150 protein 6 h after the training session. Both hippocampi of the mice were collected 6 h after training. At this timepoint AKAP150 mRNA levels showed the largest increase after LTP induction (Genin et al., 2003) and this time point corresponds to the memory consolidation phase (Igaz, Vianna, Medina, & Izquierdo, 2002). AKAP150 protein levels were measured using Western blotting. Hippocampi were homogenized at 4 °C with a plastic homogenizer, in a homogenization buffer containing 50 mM Hepes (pH 7.4), 150 mM NaCl, 0.2% NP-40, 4 mM EGTA, 10 mM EDTA, 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM sodium fluoride, 5 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM PMSF, and Complete Mini Protease Inhibitor Cocktail (Roche). The insoluble material was removed by centrifugation at 20,000*g* for 10 min at 4 °C, and the resulting supernatant was assayed for protein concentration. Equal amounts of protein for each group were separated on a 10% SDS gel and transferred to an Immobilon-P membrane (Millipore Corporation, Bedford, MA, USA). The blot was probed using an AKAP150 antibody (1: 2.500; sc-6445 Santa Cruz, CA, USA) and an anti-actin antibody (1:40,000; MP biomedicals, Irvine, CA, USA). Western blots were developed using the chemiluminescence method. Immunoreactive bands were digitized and quantified using a Quantimet 500 image analysis system (Leica, Cambridge, UK).

Statistical comparisons were made by analysis of variance (ANOVA). Data were expressed as means ± SEM. Significance was determined at the level of $p < 0.05$.

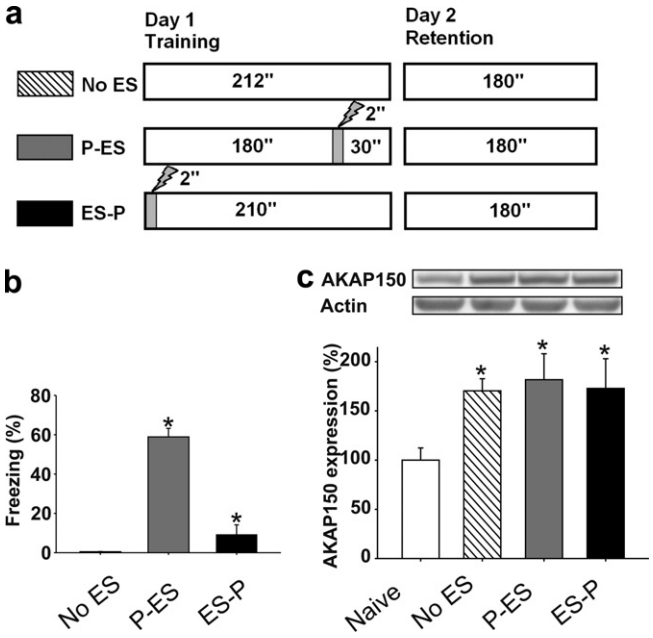

Fig. 1. Exposure to a novel context results in an increase in AKAP150 expression. (a) Schematic diagram of the behavioral protocols used for the three experimental groups. (b) Freezing behavior measured in the memory test performed 24 h after training. Statistically significant differences: $^{*}p < 0.05$ versus no-ES group. (c) Representative Western blot of four independent experiments. The bar graph summarizes the Western blot data and shows the levels of AKAP150 protein as a percentage of naive animals (set to 100%). Statistically significant differences: $^{*}p < 0.05$ versus naive.

There was no significant difference between the three experimental groups in activity during training (data not shown). During the retention test, mice of the no-ES group did not exhibit any fear-related behavior, as indicated by no freezing after re-exposure to the context (Fig. 1b). Mice that received an immediate footshock during training (ES-P) showed low freezing scores in the retention test. However, mice of the P-ES group had significantly higher freezing scores, than mice of the no-ES or ES-P groups upon re-exposure to the context (Fig. 1b). This finding was in full agreement with previous studies (Fanselow, 1980) and indicated that under entirely non-associative conditions, the shock itself did not produce a strong freezing response upon subsequent re-exposure of the mice to the chamber used in the training phase. The finding that mice exposed to context paired with shock exhibited significantly more freezing than mice exposed to immediate shock or to context only, demonstrated that the freezing behavior was induced by associative learning and did not represent an unconditional or non-associative response to the footshock employed.

A seperate set of mice was subjected to the training (No-ES, P-ES, ES-P, $n = 7$–8 per group) trials described above and together with a group of naive mice sacrificed 6 h after training. Their hippocampi were used for Western blotting to assess AKAP150 expression levels. Mice subjected to the three different training protocols all showed a strong increase of AKAP150 protein compared to naive animals (Fig. 1c). Thus, it appeared that upregulation of AKAP150 resulted from exposure of the animals to the novel environment.

To prevent any interference of novel stimuli on AKAP150 expression, in the next set of experiments, the training was preceded by a habituation procedure to completely familiarize the mice with the stimuli of the experimental set-up. Habituation consisted of a 5 min exposure to the conditioning box for three trials per day on two consecutive days (Fig. 3a). Although animals showed a decrease in activity (Fig. 2) during habituation, animals never showed any freezing behavior during these sessions.

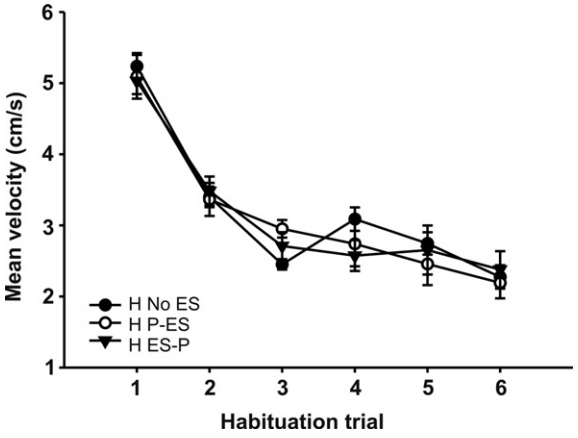

Fig. 2. Habituation causes a decrease in activity. The graph shows the time course of animal activity during the six habituation sessions.

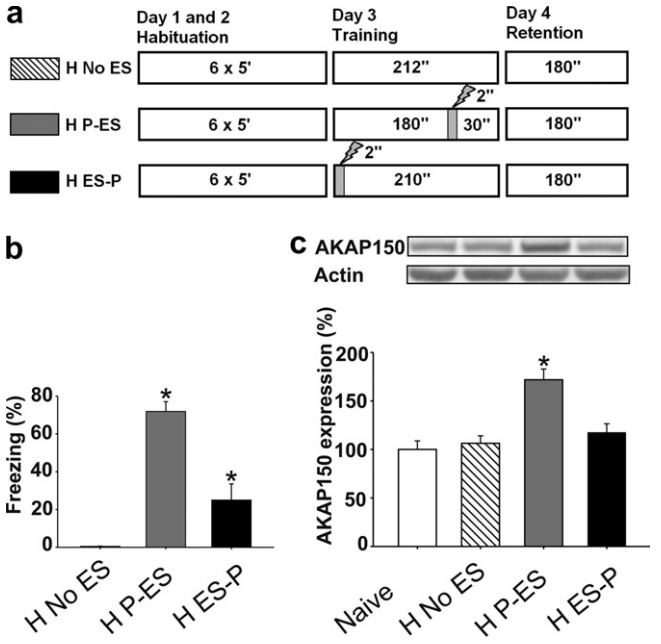

Fig. 3. Associative learning is associated with an upregulation of AKAP150. (a) Experimental paradigm for the fear conditioning tests with the training and memory test sequences preceded by a six trial habituation procedure. (b) Freezing behavior measured in the memory test performed 24 h after training. Statistically significant differences: $^{*}p < 0.05$ versus no-ES group. (c) Results are representative of five Western blot experiments. Statistically significant differences: $^{*}p < 0.05$ versus naive.

Habituation did not significantly affect the freezing behavior in the retention test. Animals that did not receive a footshock during the training (H No-ES) or were immediately exposed to the footshock (H ES-P) showed no freezing and moderate freezing, respectively, whereas strong fear-related behavior was observed in the associative learning group (H P-ES) (Fig. 3b). When training was preceded by habituation, only associative learning was paralleled by an increase in AKAP150 expression in the hippocampus 6 h after training (Fig. 3c). By exposing the animals repeatedly to the context, the novelty effect wears off as indicated by AKAP150 proteins levels in the H No-ES group that were comparable to naive animals. Habituated animals that received a footshock immediately after exposure to the box showed no difference in AKAP150 expression compared to naive animals. Thus it can be concluded that shock exposure by itself does not lead to an upregulation of AKAP150.

In summary, we conclude that both exposure to a novel context and associative learning upregulated AKAP150 expression in mouse hippocampus.

Recent research has shown that exposure to a novel event triggers a cascade of neural events that is also relevant to learning and memory. The hippocampus is an essential component of the network that detects and responds to novel stimuli (Knight, 1996). The upregulation of AKAP150 may be involved in this processing of novelty detection or may be closely related to arousal and anxiety induced by the experimental conditions. In addition, we

cannot rule out the possibility that the increased AKAP150 is, in fact, related to the formation of some type of associative new learning triggered by the novel environment.

Habituation to the conditioning box resulted in a decrement of spatial exploration during successive exposures. Behavioral habituation to a novel environment is one of the most elementary forms of non-associative learning. In our experiments non-associative learning was not paralleled by an increase in AKAP150 expression in the hippocampus.

Since AKAP150 is an important coordinator of cAMP pathways (Dell'Acqua et al., 2006), it is interesting to note that non-associative learning does not involve cAMP signaling pathways. It is also not impaired by the inhibition of protein synthesis in the hippocampus (Vianna et al., 2000).

In contrast, habituated animals showed an associative learning-specific increase in AKAP150 levels in the hippocampus 6 h after fear conditioning. Long-term formation of a hippocampal-dependent form of contextual fear conditioning was shown to depend on two consolidation periods during which hippocampal gene expression is critical: around the time of training and 3–6 h after training (Igaz et al., 2002). Whereas the first period of protein synthesis involves enhanced expression of immediately early genes and transcription factors, the second period concerns enhanced expression of structural proteins. This late phase of memory consolidation of fear motivated associative learning in rats is critically dependent on cAMP signaling pathways. It was found that PKA activity is enhanced in the hippocampal CA1 region (Bernabeu et al., 1997). In addition, extracellular regulated kinase/mitogen-activated protein (ERK/MAP) kinase is necessary for the consolidation of associative memories in the mammalian nervous system (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998). Coactivation of PKA and MAPK signaling leads to the concurrent activation of CREB-dependent gene expression required for hippocampal long-term memory formation (Impey et al., 1998).

Our findings suggest a role for AKAP150 in the second consolidation period of long-term memory. Since AKAP150 levels were only measured 6 h after training we can not exclude that AKAP150 may also be important during other stages of the memory process.

In general, the upregulation of AKAP150 may be the result of de novo protein synthesis or decreased protein degradation. Although we can only speculate on the possible role of the increased expression of AKAP150, it might very well be that elevated AKAP150 levels results in a more efficient propagation of signals carried by locally generated cyclic AMP (Colledge et al., 2000; Feliciello, Li, Avvedimento, Gottesman, & Rubin, 1997), which in turn may contribute to processing the exposure to a novel context and the consolidation of associative memory.

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